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BOLTING IN SUGAR BEET (*Beta vulgaris* subsp. *vulgaris* var. *altissima* Döll): TRIGGERING, GENETIC MECHANISMS AND PREVENTION (review)

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Abstract

Sugar beet is a significant crop that is used in the production of sugar, alcohol, livestock feed, confectionery, etc. Sugar beet is a biennial plant that form a root-crop in the first year. In the second year, after winter storage, it produces a bolt with inflorescences. Bolting refers to the ability to form both peduncle and flowers within the first year of life. The formation of bolts in sugar beets is triggered by vernalization (exposure to low positive temperatures) and long daylight hours. Flowering is significant in beet-growing regions with cold springs and long daylight hours as it can result in reduced yield and sugar content. From a genetic perspective, flowering is controlled by a complex system of genes that regulate the transition from the vegetative phase to the generative phase of development. The interaction between the *BvBTC1* and *BvBBX19* genes plays a central role in this process. The functional products of these genes stimulate the expression of the flowering inducer gene *BvFT2* and inhibit the expression of the flowering repressor gene *BvFT1* (N. Dally et al., 2018). In the beet genome, several *Arabidopsis* orthologue flowering genes have been identified. These genes are characterized by differential expression and methylation, which are influenced by vernalization and vary between flowering-resistant and non-flowering genotypes (M.-V. Trap-Gentil et al., 2011; Z. Pi et al., 2021). The main physiological regulator of flowering in sugar beets is gibberellic acid, which is also involved in vernalization through the regulation of synthesis regulator genes (E. Mutasa-Gottgens et al., 2009). The primary methods for controlling flowering involve implementing suitable agrotechnical practices and developing resistant varieties and hybrids through breeding and genetic techniques. Agrotechnical practices include selecting the appropriate sowing date to avoid exposing plants to low temperatures, choosing recommended varieties for the cultivation zone, removing early flowering plants, and using chemical treatments on seeds and vegetative plants (I.A. Oksenenko et al., 1987; K.S. Devlikamov et al., 2016; M. Sadeghi-Shoae et al., 2017). Breeding methods involve creating an analytical framework for the negative selection of flowering material. This includes practices such as ultra-early and sub-winter sowing, selection under long-day conditions, sowing with vernalized seeds, and sowing in soil treated with herbicides (A.V. Kornienko et al., 1983; A.V. Logvinov et al., 2021, 2022). It is crucial to assess genetic collections from global repositories of cultivated and wild accessions in order to identify new sources of resistance to flowering (E.S. Kutnyakhova et al., 2016; V.I. Burenin et al., 2018). An important method for generating new non-flowering alleles is mutagenesis using ethyl methanesulfonate. Markers for allelic variants (haplotypes) of functional flowering genes, as well as quantitative trait loci and single-nucleotide polymorphisms associated with resistance to bolting can be used in marker-assisted selection (B. Büttner et al., 2010; Y. Kuroda et al., 2019; S. Ravi et al., 2021). Great prospects for accelerated sugar beet selection and seed production can be achieved through the "seed to seed" scheme. This involves stimulation of bolting under artificial climate conditions by carefully controlled growing parameters, including the vernalization stage. Important parameters for successful vernalization are temperature, the phenophase of vernalization initiation, and the duration of the photoperiod.

Keywords: sugar beet, vernalization, flowering, bolting, selection, marker-mediated selection, gene networks, agricultural technology, accelerated selection

Sugar beet (*Beta vulgaris* subsp. *vulgaris* var. *altissima* Dull) is a universal industrial agricultural crop. Despite the fact that the only purpose of its cultivation is to obtain sugar from root crops, the plant is processed with virtually no waste. Thus, the tops remaining during harvesting are placed in the soil as organic fertilizer and sent to feed cattle and pigs; molasses is used in confectionery, in the production of yeast, alcohol, citric acid, etc.; pulp and molasses are also used for feed; defecate can be used as lime fertilizer [1].

Sugar beets are of great importance in crop rotation as a precursor for corn, legumes, annual grasses, millet, and early spring grains, which produce higher yields due to the soil-improving and phytosanitary positive effects of sugar beets [2]. However, the value of sugar beet as a precursor depends on climatic and soil conditions.

Sugar beets are divided into three groups, the high-yield (large root crops with a low sucrose content), high-yield-sugar (medium-sized root vegetables with an average sucrose content) and sugary (relatively small root vegetables with increased accumulation of sucrose). Thanks to the work of breeders, the sugar content in beet roots has risen from 1.3% from the time it was discovered in the roots to 17-20% [3, 4].

In 2017-2022, the world produced an average of 275 million tons of sugar beet annually, with a total planted area of about 4.6 million hectares [5, 6]. For this period statistics show that the European Union as a whole can be called a leader in the production of sugar beets with average gross harvest of approximately 113 million tons from an average sown area of 1.5 million hectares. However, when analyzing the each state separately, the Russian Federation demonstrates primacy in the industry with average gross harvest of approximately 44 million tons from an average sown area of 1.1 million hectares [5-7]. In France over the same period, the annual harvest of this root crop was approximately 36 million tons, in Germany and the USA approximately 30 million tons [6]. Sugar beets account for approximately 20% of world sugar production [5], and in Russia, this crop remains the only source of domestic raw materials for sugar production [8].

In 2022-2023 in Russia, sugar beets were grown in 24 regions and processed at 65 sugar factories in 18 regions. The gross harvest of sugar beets in the Russian Federation increased by 15% and returned to the values of the first post-Soviet peak in 2011 [9]. The most favorable regions for the crop are chernozems in the south of Russia, e.g., Krasnodar Territory, Volga region, Chernozem region, the North Caucasus and the Volga region.

One of the pressing problems of field commercial two-year cultivation of the crop is the so-called bolting, that is, flowering in the first year of the plant's life. Flowering is necessary to produce beet seeds, but when growing root crops, the varieties should not enter this stage of development.

Our goal was a systematic review of publications on bolting, the causes of its occurrence and methods to avoid the problem in sugar beets.

Tasks and challenges of sugar beet breeding and seed production in Russia. Sugar is an irreplaceable resource of quickly accessible energy for humans. As the world's population grows, the global demand for sugar also increases. Despite the fact that the sugar beet sown area in the Russian Federation is the largest in the world, the harvest here is several times less than in countries with smaller sown areas. On average for 2017-2022, the yield of sugar beet in Russia was 425 c/ha vs. 812 c/ha in France, 745 c/ha in Germany, 735 c/ha on average in the European Union, 682 c/ha in the USA, that is, approximately 1.5-2 times higher than in Russia [5-7].

In addition, with Russia's leading position in the area of sugar beet crops in recent years, more than 90% are sown with imported seeds [10]. Among other reons,

this is due to progressive agricultural technologies that exclude manual labor, and the resulting seed quality, i.e., grinding, calibration, pelleting, germination approaching one hundred percent, etc. [11].

However, despite the fact that the yield of imported hybrids and varieties is higher than that of domestic ones, it turned out that foreign hybrids are susceptible to diseases, such as root rot under conditions of excessive moisture, and do not accumulate the percentage of sugar content declared by their manufacturers. Domestic varieties and hybrids, compared to foreign ones, are more resistant to abiotic and biotic stress factors of the environment and have better shelf life, that is, they have less sugar loss during storage [12, 13]. This breeding potential must be used, so the lag in the methodology of scientific research and the use of breeding and seed advances are unacceptable, since this will limit the dependence of our farms on foreign seed suppliers, negatively affecting the economic and technological sustainability of the country's beet-sugar complex [10, 14]. In 2016, out of 33 new hybrids in the State Register of Breeding Achievements, only three were domestic. These varieties are Azimuth bred at the Pervomayskaya selection and experimental station of sugar beet (Krasnodar Territory) for the Kuban region with average yield of 496 c/ha, Konkurs bred at the Lgov experimental breeding station (Kursk Province) for the Central Black Earth region with a yield of 421 c/ha, and RMS 127 bred at the Mazlumov All-Russian Research Institute of Sugar Beet (Voronezh Province) with a yield of 324 to 720 c/ha depending on the cultivation area [15].

In Russia, within the framework of the Federal Scientific and Technical Program for the Development of Agriculture for 2017-2025, the program "Development of selection and seed production of sugar beets" is being implemented. This state program focuses on breeding high-yielding and high-quality hybrids resistant to local abiotic stresses and diseases, on effective sugar beet cultivation, storage and processing, and on providing with high-quality seeds to reduce dependence on foreign hybrids. When creating single-seeded hybrids based on cytoplasmic male sterility (CMS), it is important to reach multi-germinate varieties and hybrids in terms of seed shape, germination energy and field germination [16-18]. Agrochemically active sugar beet varieties will produce more organic matter per unit of fertilizer applied [19]. Tolerance of hybrids to a specific herbicide will limit the herbicide use [20], and tolerance to stressful acidic soils, drought and heavy metals will expand the crop cultivation area [21, 22]. Wild beet relatives are involved in crosses as donors of valuable traits to create varieties resistant to diseases and unfavorable conditions [23, 24]. Important tools that help improve the sugar beet breeding are the molecular markers for genotyping lines and hybrids [25]. We need hybrids with a wide geographical area of cultivation, including in the northern regions, that is, cold resistant, with high productivity, product quality and not prone to bolting [26].

Bolting of sugar beets. Sugar beet has a two-year life cycle. In the first year it forms vegetative organs (shortened stem, root crop, leaves), in the second year a peduncle with seeds appears. In sugar beet traditional commercial breeding, non-planting, planting and transplanting (steckling planting) methods have become widespread to produce seeds. In the first year of life, mother roots (mother plants) are grown from the sown seeds, and in the second year of life, the seed yield is obtained [11]. Typically, leaves and flowering shoots grow in root crops planted in the soil in the second year of the growing season from dormant axillary buds formed in the first year at high temperatures. A further decrease in temperature to 0...+8 °C provokes the development of the latter. Under natural conditions, this decrease occurs in winter, and in the spring of the second year, the buds produce flowering shoots. However, in some cases, this can occur in the first year of plant life, which leads to bolting.

For every 1% of bolting plants, there is a reduction in yield by 0.5-0.7% [27]. In the root crops of bolting beets, the cell walls are compacted with an increased amount of lignin. Such root vegetables are difficult to cut into shavings due to excess fibrousness. The root crops of bolting beets differ significantly from ordinary ones not only in their chemical composition, but also in the increased mass of the head (20-22% of the root crop mass vs. 11-13%), increased woodiness and the content of molasses-forming substances, reduced sugar content and lower purity of beet juice. Bolting beets, especially early ones, are more susceptible to diseases and are unstable during storage due to greater damage by black rot [28-30].

In the fields, even a seemingly insignificant number of bolting plants can create big problems. Particularly dangerous is early bolting which becomes the precursor of a malicious weed, the weedy (wild) beets, since the fallen seeds can remain in the soil for decades without loss of germination ability. Seeds that have undergone vernalization germinate randomly in rows and between rows, bolt in 100% of cases, form numerous seed plants, and again repeatedly weed the fields, winning the fight for nutrients and inhibiting cultivated beet plants. This leads to yield losses and can paralyze the sugar mills. In weed-infested fields, specific beet diseases and pests spread [31].

Studies in model plants have shown that the regulation of flowering involves multiple pathways that depend on both environmental and endogenous signals [32]. The causes of bolting can be external (e.g., due to the influence of temperature and light conditions, mineral nutrition, herbicides, etc.) and internal, the genetically determined.

External factors for bolting. The bolting of sugar beets can be caused by very early sowing, prolonged low-temperature influence, the so-called vernalization (0...+10 °C for 1-6 weeks, often in lowlands, especially when cotyledon leaves and the true leaves appear) [33], illumination for more than 12 h, and depending on quality and intensity of illumination [34].

One of the main reasons for beet bolting is sowing too early, prolonged cold springs with a sharp cold snap without precipitation. In this case, the sown seeds lie in the ground for up to 40 days and manage to go through the stage of vernalization, i.e., acquiring or accelerating the ability to shoot and flower as a result of a long stay at low temperatures. For example, in 1974 in the Vinnitsa region of Ukraine, spring was dry and cold. The field was sown with beets on March 28 and partially reseeded on May 13 due to poor seedlings. During harvesting, 27% of beet plants in the early sowing area were bolting, while there was none at all in the reseeded areas [28].

According to the All-Union Research Institute of Sugar Beet [2], with the spread of crops to the north, bolting increases due to lower air temperatures, lengthening daylight hours and far red spectrum of light with long waves which provokes shadow avoidance syndrome, when the stem sharply elongates towards the light source. For example, when sowing the same beet variety in the Vologda Province with daylight period of 20 h 05 min in June, and in Kyrgyzstan with 15 h 10 min daylight period, the bolting rate was 10.2 and 0.01%, respectively [28].

Several researchers point to a connection between plant growth and premature bolting. For example, there is a positive correlation between the proportion of bolting plants and soil fertility or between the proportion of bolting plants and watering. In addition, a connection has been noted between the use of fertilizers, herbicides, mineral nutrition [35, 36], especially after vernalization, and the development of flower stalks, with nitrogen fertilizers having the strongest effect [28]. Reduced plant density has also been reported to result in more bolting plants [37]. It seems that favorable growing conditions, especially after vernalization, promote premature

bolting of sugar beets, with bolting being more pronounced at higher yields [36, 38]. Although there are several indications of a relationship between rapid growth and bolting, growers are unlikely to limit flowering by radically changing growing practices, since anti-flowering measures may reduce yields.

It is important to note that in addition to vernalization, the phenomenon of devernalization has been established. In years with the return of spring frosts after emergence, but with the subsequent rapid onset of a warm period, the percentage of bolting plants turned out to be lower than in years when there were no frosts, but the plants were exposed to low temperatures for a long time [36].

Single-seeded beet varieties and hybrids have lower cold resistance and, therefore, higher bolting rate than multi-seeded varieties [28]. The most bolting are single-seeded tetraploid plants, followed by single-seeded diploid plants, multi-seeded tetraploid plants. In the literature, along with the concept of single- and multi-seeded plant, there are the terms dioecious and monoecious, single- and multi-sprouted forms. Multi-seeded diploid varieties are the least prone to bolting due to more focused elaborated breeding [18]. In addition, the size of fruitlets influences the bolting rate. Large ones appear earlier, and, therefore, produce the seedlings faster providing their longer vernalization [38].

Mathematical models for bolting prediction. Predicting the percentage of bolting plants in crops depending on external factors, e.g., daylight hours and temperature that largely depend on the sowing date, is important for planning agrotechnical measures. Planting time can influence sugar content, yield, and harvest time, which in turn is related to sugar mill operations [39-41].

In the UK, a “cool day” model was used to determine the expected proportion of bolting sugar beet plants in a field, in which the percentage of bolting plants is explained via the number of days with a maximum air temperature of less than 12 °C [27].

Later G.F.J. Milford et al. [34] proposed the equation to calculate the correction factor for the duration of vernalization (vernalization weighting) (1):

$$y = -1.256 + (1.260 + 0.131x) \cdot 0.9357^x, \quad (1)$$

where y is the correction factor for the duration of exposure by which the time (hours) of vernalization is multiplied; y depends on x , the observed temperature at a particular hour [34].

Typically, a massive transition of plants to bolting in a plot or in a growing season occurs after a certain threshold value of the sum of weighted vernalization hours, when the proportion of bolting plants increases sharply. This parameter is called “vernalization requirement” (VR).

The expected proportion of bolting plants (y) is determined according to equations (2) and (3):

$$y = 0 \text{ when } VI \leq VR, \quad (2)$$

$$y = BS (VI \cdot VR) \text{ when } VI > VR, \quad (3)$$

where BS (bolting sensitivity) is the proportional increase in bolting plants with each 10-hour increase in above-threshold vernalization, VI (vernalization intensity) is the accumulated number of weighted hours of vernalization between sowing and the end of June.

T. Chiurugwi et al. [42] used this model to determine the earliest time for sowing sugar beet in the UK that would have a 95% chance of avoiding bolting. Note that to use the model in Russia, recalculation of indicators is necessary. In addition, the model requires the inclusion of new coefficients, such as daylight hours, so that it can be used for both field forecasts and controlled climate chamber experiments.

E. Mutasa-Gottgens et al. [43] determined the bolting time as a function of

the height of the peduncle and the thermal time accumulation at a temperature threshold of 3 °C as the number of days after vernalization with a temperature above 3 °C multiplied by the average temperature per day. They used the equation (4), developed by J. Goudriaan et al. [44]:

$$H = (c/r)\ln(1 + \exp[r(\theta - \theta_b)]), \quad (4)$$

where H is the height of the peduncle, θ is the thermal time accumulation since the end of vernalization, r is the initial relative growth rate, c is the maximum absolute growth rate, θ_b is the accumulated thermal time at which the peduncle transition from exponential to linear growth occurs.

The model can be useful in experiments with different genotypes to determine the thermal time required for bolting after vernalization.

Internal factors for the occurrence of bolting. The genetic control of bolting in sugar beets is complex and has not yet been fully studied, despite a significant amount of data from various groups of researchers. To date, several models of gene networks regulating bolting have been proposed, in which, in addition to genes and protein regulators that mutually terminate or activate each other through cis- and trans-interactions, epigenetic and hormonal mechanisms are involved, triggered by external signals, such as photoperiod and vernalization.

The central gene in the genetic system of transition to flowering in sugar beet is *BvBTC1* (*BOLTING TIME CONTROL 1*), located in the B locus. *BvBTC1* belongs to the pseudoresponse regulator (*PRR*) genes and is homologous to the *Arabidopsis* gene *PSEUDO RESPONSE REGULATOR 7* (*PRR7* is the closest homologue of the sensitivity gene to photoperiodism in cereals *PPD1*). *BvBTC1* encodes a protein that carries a receiver response regulator (REC) domain [45], and photoperiodism sensitivity domains CONSTANS (CO), CONSTANS-Like, and TOC1 (CCT) [46].

Another bolting gene was cloned from the B2 locus and named *BvBBX19* (*DOUBLE B-BOX TYPE ZINC FINGER*). In the gene network, *BvBBX19* is upstream of *BvBTC1* and influences it epistatically [47]. Plants that simultaneously carry functional alleles *BvBTC1* and *BvBBX19* are characterized by a one-year life cycle. Both *BvBBX19* and *BvBTC* are homologous to the *Arabidopsis* CO protein which induces *FT* gene expression. However, unlike CO, *BvBBX19* carries two zinc finger domains (B-box) but lacks a CCT domain; the *BvBTC* protein, on the contrary, carries a CCT domain.

Two *FLOWERING LOCUS T* (*FT*) genes, *BvFT1* and *BvFT2*, belong to the phosphatidylethanolamine-binding protein (PEBP) gene family located in the gene network downstream of *BvBTC1* and *BvBBX19*, therefore, the expression of *BvFT1* and *BvFT2* is controlled by the expression products of *BvBTC1* and *BvBBX19* [47, 48]. *BvFT1* and *BvFT2* are antagonist genes, while *BvFT2* promotes flowering and is required for flower development like its *Arabidopsis* ortholog gene *FT*, *BvFT1* acts as a repressor of flowering unlike *Arabidopsis FT*). N. Dally et al. [49] suggested that the functional proteins *BvBTC* and *BvBBX19* form a heterodimer containing both CCT and B-box domains. It acquires the ability to increase the expression of the flowering inducer gene *BvFT2* and inhibit the expression of the flowering repressor gene *BvFT1*, which determines the annual type of development. With dysfunctional mutations, *BvBTC* and *BvBBX19* lose this ability, resulting in either a two-year phenotype or a complete loss of the ability to form a peduncle.

Based on the analysis of coexpression of multiple sugar beet genes in leaves, a two-module model was proposed to describe the plant transition to flowering [50]). The first module includes four genes of the photoperiodic pathway (*BvELF3*, *BvGI*, *BvTOC1* and *BvBOA*), three genes of the autonomous pathway (*BvFVE1*, *BvFLD* and *BvFCA*) and *BvBTC1*. All genes serve as positive regulators of each other, with

the exception of *BvFVE1*. Its expression is negatively correlated with *BvELF3* [51]. In the second module, *BvFT1* and *BvFT2* were associated with *BvLHY*, *BvGATA22* and *BvFVE2*. *BvGATA22* showed negative feedback with the flowering activator *BvFT2* and positive feedback with the flowering inhibitor *BvFT1*. The expression of the latter was also positively correlated with the expression of *BvLHY* and *BvFVE2*.

It has also been established that sugar beet genes, the orthologs of which in *Arabidopsis* are associated with hormonal status, change their expression during vernalization and/or in genotypes resistant to bolting. Among them, there are the gibberellin pathway genes *BvGA20ox1*, *BvGA20ox2*, *BvRAV1*-like, *BvRAV1*, *BvDELLA* and *BvRGA*, as well as the cytokinin-dependent gene *BvGATA22* [42, 51-53].

In response to vernalization, differential expression of vernalization pathway genes, such as *BvVRN1*, *BvVRN1*-like, *BvVAL1*, *BvVAL2*, *BvVIN3*, occurs [54, 55]. In addition, small interfering RNA miR156 and long non-coding RNA MSTRG.26204.1 participate in vernalization [56, 5]. It has been revealed that the methyltransferases *BvDNMT* and *BvRNMT* which are factors of epigenetic modifications of DNA and RNA, respectively, are involved in vernalization [54, 58, 59]. Based on an integrated approach regarding both differential methylation and expression, a model was developed centered on the *BvBTC1*-*BvFT1*-*BvFT2* "core." The upstream flowering blocker *BvFL1* is activated by *BvRNMT* and inhibited by *BvFVE* and long-term vernalization (for 9 weeks). The activator of flowering *BvFT2* is positively regulated by *BvCOL1* and, in turn, positively regulates the expression of *BvAGL24* and *BvFUL*. *BvCOL1* and *BvBTC1* are also positively regulated by photoperiod length. Moreover, in boltin-unresistant genotypes, the *BvRNMT*, *BvFVE*, *BvFL1*, *BvFT1* and *BvFT2* genes are hypermethylated [59].

The study of lines derived from ethyl methane sulfonate (EMS)-induced mutagenesis and the natural allelic diversity of sugar beet and its wild relative, sea beet (*Beta maritima* L.), made it possible to find new loci that determine the requirements for vernalization, one- or two-year life cycle or flowering time. On chromosome II, two unlinked loci, *LB* and *LB2*, were identified, which in the recessive state form a late-flowering phenotype [60, 61]. B. Büttner et al. [62] identified two loci, *B3* and *B5* that affect flowering timing and are not linked to the *BvBTC1* locus. S.F. Abou-Elwafa et al. [63] discovered the *B4* locus, determining the requirements for vernalization, at a 11 cM distance from the *B* locus on chromosome II. Y. Kuroda et al. [64] identified the dominant gene *BLOND*, its carriers form seeds in 4 months under 24-hour daylight without vernalization. N. Pfeiffer et al. [65] identified QTL (quantitative trait loci) *BR1* on chromosome IX, associated with resistance to bolting after winter, for which C. Tränkner et al. [66] identified *BvCPSF73-Ia* as the most likely candidate gene and also identified an additional compensatory gene *BvCPSF73-Ib*.

N. Pfeiffer et al. [67] identified three QTL, on chromosomes III (*DTBnat1-DTBart1*), V (*DTBnat2-DTBart2*), and IX (*DTBnat3*) that influenced the timing of bolting transition. In beets, a tandemly duplicated locus *Bv_22330_orky* was discovered on chromosome VI, in the intron of which SNP183 (single nucleotide polymorphism) was associated with the predisposition of sugar beets to flowering [68]. Y. Kuroda [69] identified QTL *qB6* in close proximity to this SNP, associated with resistance to bolting. The author believes that it may correspond to the previously described genes *BvFL1* or *Bv_22330_orky*. S. Ravi et al. [70] found two SNPs associated with a low propensity of sugar beet to bolting. The first is SNP_36780842 on chromosome I in the 3' UTR of a gene homologous to the genes of the chaperone-J-domain superfamily which involved in the control of flowering. The second is SNP_48607347 on chromosome II in the exon 3 xylose isomerase genes, probably

involved in the modulation of the endogenous amount of sugars, important for signaling during the transition to flowering. Y. Kuroda [69] showed minor QTL associated with bolting, including *qB1* on chromosome I near QTL SNP_36780842. Minor QTLs *qB8* on chromosome VIII and *qB9* on chromosome IX were also found.

In addition to nuclear genes, the mitochondrial genes *ORF152*, *ORF102b*, *ORF192*, *ORF104*, and *COX2* have also been shown to be differentially expressed and/or methylated [59].

The transition to flowering is accompanied by complex changes in the hormonal status of the plant. Among the hormones associated with vernalization, gibberellic acid (GA) plays a significant role. This is shown in works on the influence of hormones on the transition of sugar beet plants to bolting and flowering, depending on the genotype and growing conditions, especially temperature and day length.

E.S. Mutasa-Gottgens et al. [71] demonstrated that genotypes *BB* and *Bb* require long daylight hours for bolting, regardless of gibberellin status, while biennial genotypes *bb* require vernalization for the GA-mediated transition to bolting. For transition from bolting to flowering, both genotypes require long daylight hours, and the GA content is not a limiting factor. Y. Koda et al. [72] found that exogenous jasmonic acid (JA) leads to thickening of the main and, to a greater extent, lateral roots, inhibition of bolting caused by GA treatment and vernalization. The JA content in the apical leaves of plants in the field increased during the summer, reaching a peak in August and decreasing in September. N. Liang et al. [73] demonstrated in plants grown from vernalized roots that after vernalization there is an increase in the amount of GA and indolylacetic acid, associated with the accumulation of auxin signaling protein GH3.1 and gibberellin signaling protein GA3OX1. Bolting probably occurs when a certain concentration of these hormones is reached.

Expression of the *BvRAVI-like* gene increased 2.5 times after vernalization and an additional 3 times after treatment of sugar beet plants with gibberellins. Without vernalization, treatment with gibberellins reduced the expression of this gene [53]. E.S. Mutasa-Gottgens et al. [53] identified 19 genes differentially expressed by GA treatment. According to L. Zhao et al. [52], vernalization suppresses the expression of *BvABFs* and *BvMYC2s*, implying inhibition of abscisic and jasmonic acid signaling.

Agrotechnical methods for combating bolting. The main method remains the optimal sowing time, compliance with the requirements of agricultural technology, the use of resistant hybrids and varieties in the regions according to recommendations [28]. Even the most genotypically productive hybrid will show unsatisfactory characteristics if seeds are used that are poorly prepared at the seed plant and obtained in violation of agrotechnical requirements [74].

The Russian Federation is a country with different soil and climatic conditions in each beet growing zone, that is, varieties and hybrids must meet certain specific requirements. Drought-resistant and early-ripening varieties are needed for the Central Black Earth zone, the varieties responsive to irrigation, late-ripening and resistant to cercospora are needed for the southern territories, and non-bolting varieties for the northern regions [18]. It has been established that beet varieties that were created for northern latitudes do not form bolting plants, while those produced for middle latitudes can produce up to 10% bolting plants, those originating from southern countries — 10-50%, and from the most southern and hottest countries up to 100% [36]. As a rule, timely destruction of early bolting plants in the fields during the period from bolting to budding prevents crop rotation from being clogged with wild beets. This is an effective and cheap method, similar to manual weeding on grain crops. It is also advisable to identify and consider bolting plants in trials along

with productivity assessments [31].

Several methods of chemical treatment of sugar beet seeds have been patented to combat bolting and to breed forms resistant to premature flowering. This includes treating seeds with a treflan solution with storage at +8...+10 °C and subsequent winter sowing. In the spring, bolting plants are eliminated, and non-bolting plants are preserved until the end of the growing season and are used as source material with resistance to bolting [75]. Another way is to treat the seeds with a solution of chlorocholine chloride (TUR retardant) which delays the initial formation of the seedling, as a result of which the spring development of beets occurs at a higher temperature, the number of bolting plants decreases by 1.6-4.2 times, and the yield increases. The method is recommended for northern regions [76]. The use of paclobutrazol, a plant growth regulator and gibberellic acid inhibitor, is also proposed to reduce the percentage of bolting, to increase the sugar content and to improve the quality of root crops, depending on the genotype of sugar beet [77, 78].

Breeding methods for creating varieties resistant to bolting. To select non-bolting forms, the pre-winter sowing is used when the air temperature is approximately 0 °C and the soil temperature drops to +2...+4 °C. This method is used at the Mazlumov All-Russian Research Institute of Plants and Plants in some experimental breeding stations. Another methods are ultra-early sowing; selection of vernalized seedlings under long-day (the method has been developed by N.A. Negovsky); sowing seeds vernalized for 45 to 60 days (it is especially effective in the western and northwestern regions); negative selection of early ripening plantings; selection under polar day conditions at the VIR polar station. Selection within a population is most effective [18].

A.V. Logvinov et al. [36] developed and put into practice in the conditions of the Krasnodar region reliable methods for assessing and selecting bolting-resistant initial breeding material and commercial hybrids. After pre-winter and early spring sowing dates, bolting plants were detected in June and September before harvesting. Dioecious forms exhibited bolting (mostly early) to a greater extent than monoecious forms. Vector and Atamansha hybrids showed the best resistance to bolting (0%). Further studies have demonstrated that an effective method for assessing and obtaining breeding material resistant to bolting is provocative early spring sowing with seeds germinated at +9 °C or treated with an aqueous solution of the herbicide Burefen FD-11 (emulsion concentrate, active ingredient desmedipham 80 g/l and phenmedipham 80 g/l, FSUE VNIHSZR, Russia) at a working solution concentration of 5 ml/l [36].

A comparative study and assessment of sugar beet breeding material for resistance to bolting was carried out by A.V. Logvinov et al. [30] (Pervomaisk Selection and Experimental Station of Sugar Beet, Krasnodar Territory, and Experimental Scientific Station for Sugar Beet, Republic of Belarus) using a specially developed provocative technique. Pre-winter and early spring sowing was carried out with seeds pre-soaked in water and kept for 20 days at +3 °C. the hybrids Pervomaisky and Korvet expressed the greatest resistance to bolting [30]. Sowing of vernalized germinated seeds in a greenhouse under additional lighting may be used to isolate non-bolting forms from the beet population [38]. A.V. Kornienko et al. [79] propose provocative conditions by introducing into the soil a mixture of herbicides Eptam and Lenatsil, which enhances bolting by 20%, followed by selection of non-bolting forms. To maintain the so-called 'stubborn ones' in genetic collections, a cultivation method with a multi-level rejection system has been developed [80].

Studying the genetic diversity of sugar beet allows identification of new donors of resistance to flowering. E.S. Kutnyakhova et al. [81] in 2012-2014 in evaluation of sugar beet hybrids bred by Lion Seeds Co., Ltd. (Thailand) and Mazlumov

VNISS found out that half of the samples showed bolting from 0.4 to 1%. V.I. Burinin et al. [82], when assessing the VIR collection, found that samples from Sweden were characterized by the greatest resistance to bolting, the breeders from Germany have also successfully created dioecious specimens resistant to bolting.

Marker-assisted selection of bolting-resistant plants. MAS for any trait is based either on functional markers of allelic polymorphisms of genes with known sequences, or on markers linked (associated) with traits. If in the first case the gene sequence and phenotypic manifestation of the alleles are known, then in the second case information about the structure of the gene and the functional role of the found nucleotide polymorphism is most often absent. A feature of the studied functional genes that regulate flowering in sugar beets is the presence of many polymorphisms between allelic variants, including SNPs and indels, which allows them to be called haplotypes.

B. Böttner et al. [83] developed a codominant marker, GJ1001c16, that distinguishes the dominant *BvBTC1* allele (one-year life cycle) from the recessive one. The marker has been tested in many studies with segregating populations to search for alternative vernalization genes [53, 63, 67, 84].

For the allele resulting from EMC-induced mutagenesis and leading to a two-year phenotype, the CAPS marker CAU4206 (primers NH619 + NH620 and restriction enzyme *Hinf*I) was developed [85]. Y. Kuroda et al. [86] developed primers F2/R2 to amplify the sequence between exons 7 and 9. The use of the *Hha*I restriction enzyme made it possible to distinguish between alleles *a* (biennial developmental type), *g* and *o* (one-year developmental type). In sugar beet, the allelic diversity of *BvBTC1* is well described, its nucleotide sequences are publicly available [85-88], so the development of new molecular markers of polymorphisms characteristic of certain alleles are expected. For anonymous genome regions associated with resistance to bolting, we can note the TaqMan marker of single nucleotide polymorphism SNP18. Its allelic variant *T* is associated with resistance to bolting, *C* with susceptibility [68]. Another markers are two HRM (high resolution melting) markers, the SNP /SNP_36780842 (*G* allele is associated with bolting resistance, *C* allele with susceptibility) and SNP21/SNP_48607347 (*C* allele is associated with bolting resistance, *A* allele with bolting susceptibility) [70].

Mutagenesis and genetic engineering in the creation of plants resistant to bolting. To create new highly adaptive breeding forms of sugar beet, it is necessary to expand its allelic diversity, including genes that determine the requirements for vernalization, sensitivity to long daylight hours and resistance to bolting. It is possible to create fundamentally new alleles or use new genes in the beet genome using mutagenesis, genetic engineering and genome editing.

Mutagenesis is a fundamental method for studying the structural and functional characteristics of a gene, as well as one of the available methods for increasing genetic diversity and obtaining new promising breeding forms, in particular sugar beets [89].

The TILLING method (the targeting-induced local lesions in genomes) is based on point mutations using EMS-induced mutagenesis with subsequent identification of the target gene in the resulting lines by the reverse genetics method [90].

U. Hohmann et al. [91] used EMS-induced mutagenesis to create a collection of sugar beet lines based on the early flowering line 930190. Experiments with mutant lines identified loci *B2*, *B3*, *B4*, and *B5* [62, 63, 85]. A model of the interaction between the *BvBTC1* and *BvBBX19* proteins was constructed [47, 49], and a new allele *BvBBX19h* was obtained [49]. S.L. Frerichmann et al. [92] using the EcoTILLING method with restriction enzyme *CELI* to search for mutations, detected 20 silent SNPs and one nonsynonymous SNP in the *BTC1*, *BvFL1*, and

BvFT1 genes, resulting in 55 haplotypes. The authors also found associations of nucleotide polymorphism in *BvFL1* with winter bolting and winter hardiness.

Another approach to improving sugar beets is the creation of transgenic plants. In order to study the influence of hormonal status on bolting and flowering, E. Mutasa-Gottgens et al. [43] obtained transgenic lines of sugar beet with genes for hormonal metabolism of beans and *Arabidopsis*. A transgenic sugar beet line with the bean gene *PcGA2ox1*, which is involved in the degradation of biologically active forms of GA, required an additional 20 days for the transition to bolting, had a dwarf phenotype and was sterile, but male fertility was restored by spraying with GA. The *Arabidopsis* transgene *gai*, which is an allelic variant of the DELLA protein lacking the DELLA domain and weakly sensitive to GA, caused a delay in bolting in a sugar beet plant for 11-14 days while maintaining fertility [43].

Genome editing allows new alleles to be created based on existing genes, and the resulting plants do not carry transgenes. To date, there is only one report of CRISPR/Cas9 editing of sugar beet in relation to resistance to beet curly top virus [93]. Since allelic variants that lead to the formation of a two-year phenotype requiring vernalization arise as a result of disruption of the functionality of proteins involved in the transition to bolting and flowering, genome editing as a tool for obtaining non-functional alleles is promising for the creation of sugar beet forms resistant to bolting.

To summarize, it should be noted that beet growing remains one of the most popular, knowledge-intensive, technologically and organizationally complex industries. The problem of import substitution of seed and varietal material of sugar beet requires effective interaction between representatives of various scientific fields. The combination of modern agrotechnical, biotechnological, molecular genetic methods (including genomics and epigenomics, transcriptomics, metabolomics and proteomics), speed breeding technologies and classical selection methods in the creation and cultivation of sugar beet hybrids will increase their productivity and the quality of domestic seed material. Vernalization of seeds in the mother plant and the biotechnological method of rescuing embryos seem to be promising methods.

Thus, bolting is a problem faced by many sugar beet growers. The most effective solution is to obtain genotypes in which resistance to bolting is combined with a complex of other useful traits, e.g., productivity, sugar content, resistance to abiotic and biotic stresses during the growing season, di- and monoecious forms, long-term storage, technological qualities, etc.). The use of varieties recommended for a specific zone and compliance with the regulations for their cultivation, including seed treatment, allows avoiding plant bolting. Bolting is a complex natural phenomenon, the physiological and molecular genetic mechanisms of which continue to be studied. Their understanding and assessment of genetic collections of cultivated varieties and wild species will allow us to identify and obtain new alleles for resistance to bolting. When creating haplotypes suitable for selection, genomic editing can be used along with classical mutagenesis. It is necessary to continue studying the allelic diversity of genes regulating the transition to flowering and the search for valuable nucleotide polymorphisms using genomic selection. Particular attention should be paid to the speed breeding to obtain seeds under controlled conditions due to the ability of sugar beets to form a flowering shoot from a rosette under the influence of vernalization and long daylight hours.

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